

What is claimed is:

1. A method of preparing a nucleic acid comprising:

increasing the relative percentage of a population of nucleic acids of interest

5 within a mixed population of nucleic acids, wherein said population of interest comprises a plurality of nucleic acid sequences, comprising:

(a) contacting a nucleic acid sample with a bait molecule, wherein said bait molecule is capable of complexing specifically to a target sequence, but not to said sequences in said population of interest, under such conditions as to allow for the formation of a bait:target complex;

(b) removing said bait:target complex from said mixed population thereby resulting in an increase in the relative percentage of said population of interest;

fragmenting the sequences from said population of interest to produce fragments;

15 and

adding a signal moiety to the fragments.

2. The method of claim 1 wherein the nucleic acid sample is an RNA sample.

20 3. The method of claim 1 wherein the nucleic acid sample is derived from a prokaryotic organism.

4. The method of claim 1 wherein the nucleic acid sample is derived from a gram negative prokaryotic organism.

25 5. The method of claim 1 wherein the nucleic acid sample is derived from *E. coli*.

6. The method of claim 1 wherein said population of interest is messenger RNA (mRNA.)

30 7. The method of claim 1 wherein said target sequence is stable RNA.

8. The method of claim 1 wherein said target sequence is ribosomal RNA (rRNA).

9. The method of claim 1 wherein said target sequence is 23S RNA.

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10. The method of claim 1 wherein said target sequence is 16S RNA.

11. The method of claim 1 wherein said bait molecule is generated exogenously.

10 12. The method of claim 1 wherein said bait molecule is chemically synthesized.

13. The method of claim 1 wherein said bait molecule is cloned from single stranded phage DNA.

15 14. The method of claim 1 wherein said bait molecule is synthesized by reverse transcriptase using said target sequence as a template.

15. The method of claim 1 wherein the nucleic acid sample is an RNA sample, the bait molecule is DNA, and the bait:target complex is a DNA:RNA hybrid.

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16. The method of claim 14 wherein said bait molecules are synthesized by reverse transcriptase after the addition of primers comprising at least one of the following sequences:

5'-CCTACGGTTACCTTGTT-3'

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5'-TTAACCTTGCGGCCGTA CTC-3'

5'-TCGATTAACGCTTG CACCC-3'

5'-CCTCACGGTTCATTAGT-3'

5'-CCATTATACAAAAGGTAC-3'

5'-CTATAGTAAAGGTT CACGGG-3'

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5'-TCGTCATCACGCCTCAGCCT-3'

5'-TCCCACATCGTTTCCCAC-3'.

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17. The method of claim 1 wherein said bait is attached to a solid substrate.

18. The method of claim 17 wherein said solid substrate is a bead.

5 19. The method of ~~claim 17~~ wherein said step of removing said target sequence is accomplished by separating said solid substrate from said mixed population.

20. The method of claim 1 wherein said bait is modified to comprise a selectable element.

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21. The method of claim 20 wherein said selectable element is selected from the group consisting of: a nucleic acid sequence, a ligand, a receptor, an antibody, a haptenic group, an antigen, an enzyme or an enzyme inhibitor.

15 22. The method of claim 20 further comprising the step of exposing said bait:target complex to a reagent capable of binding said selectable element to form a reagent:bait:target complex.

20 23. The method of claim 22 wherein the reagent capable of binding said selectable element is selected from the group consisting of: a nucleic acid sequence, a ligand, a receptor, an antibody, a haptenic group, an antigen, an enzyme or an enzyme inhibitor.

24. The method of claim 20 wherein said selectable element is a biotin.

25 25. The method of claim 22 wherein said reagent capable of binding said selectable element is streptavidin.

26. The method of claim 22 wherein said step of removing said RNA sequence is accomplished by separating said reagent:bait:target complex from said mixed population.

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27. The method of claim 26 wherein the reagent:bait:target complex is attached to a solid support.

28. The method of claim 15 wherein said step of removing said RNA:DNA hybrid comprises exposing said RNA:DNA hybrid to a reagent which specifically recognizes RNA:DNA hybrids.

5 29. The method of claim 28 wherein said reagent is RNase H.

30. The method of claim 28 wherein said reagent is an antibody.

10 31. The method of claim 1 wherein the step of removing said bait:target complex is a two step process in which the target is removed first and the bait molecule is removed thereafter.

15 32. The method of claim 29 further comprising the step of removing any remaining DNA bait molecules after said target RNA sequence is removed.

33. The method of claim 32 wherein said step of removing said DNA bait molecule is accomplished by digestion with DNase I.

20 34. The method of claim 31 wherein steps (a) and (b) are repeated.

35. The method of claim 34 wherein the same bait molecule is used to remove multiple target sequences.

25 36. The method of claim 35 wherein a thermostable RNase H is used to remove said target sequences from said bait:target complex.

37. The method of claim 34 wherein step (a) is performed at a first temperature and step (b) is performed at a second temperature.

30 38. The method of claim 1 wherein said signal moiety is a biotin.

39. The method of claim 1 wherein said signal moiety is a PEO-Iodoacetyl Biotin.

40. The method of claim 1 wherein the signal moiety is attached to the 5' ends of said fragments.

41. The method of claim 40 wherein after said step of fragmenting, said 5' ends of
5 said fragments are chemically modified.

42. The method of claim 41 wherein the 5' ends of said fragments are chemically modified by (-S-ATP and T4 kinase.

10 43. The method of claim 40 wherein said chemical modification results in the addition of a thiol group to the 5' end of said fragments.

44. The method of claim 43 wherein said detectable signal moiety is PEO-Iodoacetyl Biotin.

15 45. A method of increasing the relative percentage of a nucleic acid population of interest within a mixed population of nucleic acids, wherein said population of interest comprises a plurality of nucleic acid sequences, comprising:

20 (a) contacting a nucleic acid sample with a bait molecule, wherein said bait molecule is capable of hybridizing specifically to a target sequence but not to said sequences in said population of interest, under such conditions as to allow for the formation of a bait:target complex; and

25 (b) removing said bait:target complex from said mixed population thereby resulting in an increase in the relative percentage of said nucleic acid population of interest.

46. The method of claim 45 wherein the nucleic acid sample is an RNA sample.

30 47. The method of claim 45 wherein the nucleic acid sample is derived from a prokaryotic organism.

48. The method of claim 45 wherein the nucleic acid sample is derived from a gram negative prokaryotic organism

5 49. The method of claim 45 wherein the nucleic acid sample is derived from *E. coli*.

50. A compound having the formula:

n-S-acetyl-PEO-sig

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wherein n is a polynucleotide, S is thiol, acetyl is an acetyl functional group, PEO is polyethelene oxide, and sig is a signal moiety.

51. The compound of claim 50 wherein said signal moiety is a biotin.

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52. The compound of claim 50 wherein said polynucleotide is a DNA.

53. The compound of claim 50 wherein said polynucleotide is an RNA.

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54. The compound of claim 50 wherein said polynucleotide is an mRNA.

55. The compound of claim 50 wherein said thiol group is at the 5' of said polynucleotide.

25 56. A method for labeling a polynucleotide comprising:
contacting said polynucleotide with PEO-iodoacetyl conjugated to a signal moiety
under conditions such that the PEO-iodoacetyl will attach to said polynucleotide.

57. The method of claim 56 wherein said polynucleotide comprises a thiol group.

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58. The method of claim 57 wherein said thiol group is at the 5' of said polynucleotide.

59. The method of claim 58 wherein said signal moiety is a biotin.

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60. The method of claim 56 wherein said polynucleotide is a DNA.

61. The method of claim 56 wherein said polynucleotide is an RNA.

10 62. The method of claim 56 wherein said polynucleotide is an mRNA.

63. A method for labeling a polynucleotide comprising:
contacting said polynucleotide with a reactive thiol group to form a thiolated polynucleotide;
15 contacting said thiolated polynucleotide with a signal moiety capable of reacting with said thiolated polynucleotide under appropriate conditions such that said signal moiety is attached to said polynucleotide.

20 64. The method of claim 63 wherein said step of creating a thiol group comprises contacting said polynucleotide with a gamma S ATP and a kinase.

65. The method of claim 63 wherein said signal moiety is a biotin.

66. The method of claim 63 wherein said polynucleotide is a DNA.

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67. The method of claim 63 wherein said polynucleotide is an RNA.

68. The method of claim 63 wherein said polynucleotide is an mRNA.

69. A method of labeling prokaryotic mRNA comprising:

obtaining a population of RNA comprising both stable RNA and mRNA from a prokaryotic organism;

increasing the relative percentage of mRNA in said population of RNA

5 comprising the steps of;

exposing said population of RNA to a plurality of DNA bait molecules which are complementary to at least a portion of the stable RNA in said population of RNA under such conditions as to allow for the formation of DNA:RNA hybrids;

10 exposing said DNA:RNA hybrids to RNase H to remove the RNA from said RNA:DNA hybrids, producing a sample comprising of DNA and mRNA; and

exposing said sample comprising of DNA and mRNA to DNase thus increasing the relative percentage of mRNA within said population of mRNA;

fragmenting said mRNA to form mRNA fragments;

15 exposing said mRNA fragments to γ -S-ATP and T4 kinase to produce reactive thiol groups at the 5' ends of said mRNA fragments, thereby forming thiolated mRNA fragments; and

exposing said thiolated mRNA fragments to PEO-Iodoacetyl-Biotin such that a stable thio-ether bond is formed between said thiolated mRNA fragments and said PEO-Iodoacetyl-Biotin.

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